J. Helminthol. Soc. Wash.
59(1), 1992, pp. 16–21

Superimposed Infections in Golden Hamsters Infected with *Echinostoma caproni* and *Echinostoma trivolvis* (Digenea: Echinostomatidae)

JANE E. HUFFMAN,[1,3] PATRICIA M. MURPHY,[1] AND BERNARD FRIED[2]

[1] Department of Biological Sciences, East Stroudsburg University, East Stroudsburg, Pennsylvania 18301 and
[2] Department of Biology, Lafayette College, Easton, Pennsylvania 18402

ABSTRACT: Intra- and interspecific competition were studied in *Echinostoma caproni* and *Echinostoma trivolvis* infections in golden hamsters. The *E. caproni, E. trivolvis*/hamster model had a high level of compatibility using the criterion of superimposing 5 age classes of parasites. It was possible to establish 5 age classes of both species in the hamster. Intraspecific competition occurred in *E. caproni* with the 7- and 14-day-old age classes and in *E. trivolvis* with the 21-, 28-, and 35-day-old classes. The locations of the worms in concurrent infections suggested that competition between the 2 species did not occur. Worms of both species were found in clusters or in close association with each other.

KEY WORDS: Trematoda, *Echinostoma caproni, Echinostoma trivolvis,* hamster, primary infection, challenge infection, site selection.

Widespread interest involving all aspects of infections with 37-collar-spined *Echinostoma* spp. has resulted in the compilation of extensive information. Some confusion, however, exists regarding the classification of the species used in these studies. In accord with the work of Kanev (1985), our study uses the names *Echinostoma caproni* and *E. trivolvis* for 2 related species previously referred to as *E. liei* and *E. revolutum,* respectively.

Infectivity, growth, and development of *E. caproni* and *E. trivolvis* in the golden hamster were studied by Isaacson et al. (1989) and Franco et al. (1986), respectively. Clinical and pathological effects of *E. trivolvis* in golden hamsters have also been reported (Huffman et al., 1986). Huffman et al. (1988) investigated single and concurrent infections of the golden hamster with *E. trivolvis* and *E. caproni.* The establishment, survival, and fecundity in *E. caproni* infections in NMRI mice were reported by Odaibo et al. (1988).

Holmes (1990) suggested 3 major selective forces for niche restriction in intestinal helminths: specialization, reproductive efficiency, and competition. Some authors (Behnke, 1987; Christensen et al., 1987) appeared to suggest that niche restrictions are side effects of immune mechanisms, and are not related to any selective forces acting on niches as such.

The selective factor most frequently invoked in analysis of niche occupation is competition. Intraspecific competition is a major force extending niche width, with interspecific competition a major force restricting niche overlap. Negative interactions can be revealed by reductions in establishment, growth, maturation, or

[3] To whom all correspondence should be addressed.

Table 1. Percent recovery, mean number, and total number of worms recovered from superimposed infections with *E. caproni*. Four hamsters were killed at each day postinfection (PI).

	Age class—days PI				
Day PI	7	14	21	28	35
7	26				
14	8	10			
21	13	19	30		
28	10	20	21	28	
35	17	14	20	28	34
Total no. of worms	74	63	71	56	34
Mean ± SE/host	3.7 ± 1.4	3.9 ± 1.9	5.9 ± 1.8	7.0 ± 0.92	8.5 ± 0.80
Percent recovery	37	39	59	70	85

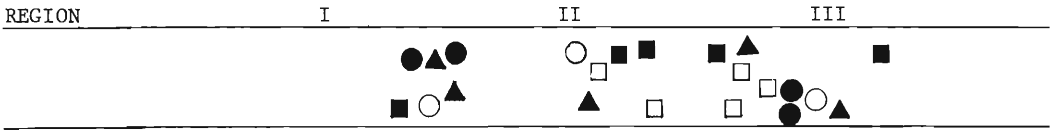

Figure 1. General distribution of the 5 age classes of *E. caproni* in the small intestine on day 7 (■), day 14 (□), day 21 (○), day 28 (●), and day 35 (▲) postinfection. Symbols represent the areas in which various age classes of parasites were recovered. Average length of the small intestine was 38 cm.

reproduction; displacement of 1 species is also common (Dobson, 1985).

Dobson (1985) noted that the extent of competition is markedly affected by intensity of the interacting species. With relatively low populations, neither intra- nor interspecific competition is likely to be significant; with relatively high populations, both are likely to be significant.

The purpose of this study was to provide evidence for: 1) the involvement of immunological reactions in superimposed *E. caproni* and *E. trivolvis* infections; 2) the site selection of 5 age classes of *E. caproni* and *E. trivolvis;* 3) the occurrence of intraspecific competition between the age classes of *E. caproni* and *E. trivolvis;* 4) the occurrence of interspecific competition between an established infection of *E. caproni* and a challenge infection with *E. trivolvis;* and 5) the occurrence of interspecific competition between an established infection of *E. trivolvis* and a challenge infection with *E. caproni.*

Materials and Methods

An outbred strain of golden hamsters (*Mesocricetus auratus*) was used in all studies. Metacercarial cysts of *E. trivolvis* and *E. caproni* were obtained from the kidney and pericardial cavities of laboratory-infected *Biomphalaria glabrata.* The metacercariae were preselected for viability.

To evaluate the occurrence of superimposed infections with either *E. trivolvis* or *E. caproni* the following protocol was used. Twenty adult male hamsters were each infected per os with 10 cysts of *E. caproni.* On day 7 postinfection (PI) 4 hamsters were killed and examined. The remaining hamsters were each infected with 10 cysts. Feces were checked for eggs on day 9 following the initial infection to confirm infection in the animals. On day 14 PI, 4 hamsters were killed and examined, the remaining 12 hamsters were again each infected with 10 cysts. This protocol was repeated on days 21, 28, and 35 PI. Twelve additional hamsters were infected and used as controls. Three animals were killed on days 14, 21, 28, and 35 PI to provide size and location data for the various age classes. It was possible to distinguish each age class based on size (length) and maturation of worms. The small intestine was then divided into 3 equal regions (I, II, and III). Worm location and percent recovery were recorded from all animals.

To evaluate the effect of a primary *E. trivolvis* infection on subsequent challenge with *E. caproni,* 15 golden hamsters were each infected with 10 metacercarial cysts of *E. trivolvis.* Feces were checked on day 9 PI. On day 14 PI the 10 infected hamsters were each infected with 10 cysts of *E. caproni.* The remaining 5 infected hamsters were designated as controls to compare parasite location. An additional 5 hamsters were each infected with 10 cysts of *E. caproni.* All animals were killed on day 28 after the primary infection. The small intestine was removed and measured from the pyloric valve to the ileocecal valve. The number of worms and their locations in the small intestine were recorded. The same protocol was used to evaluate the effect of a primary *E. caproni* infection on subsequent challenge with *E. trivolvis.*

Table 2. Percent recovery, mean number, and total number of worms recovered from superimposed infections with *E. trivolvis.* Four hamsters were killed at each day postinfection (PI).

	Age class—days PI				
Day PI	7	14	21	28	35
7	26				
14	26	36			
21	26	30	37		
28	25	33	38	31	
35	25	33	38	31	27
Total no. of worms	128	132	113	62	27
Mean ± SE/host	6.4 ± 1.3	8.3 ± 1.8	9.4 ± 0.66	7.8 ± 2.0	6.8 ± 0.95
Percent recovery	64	83	94	78	68

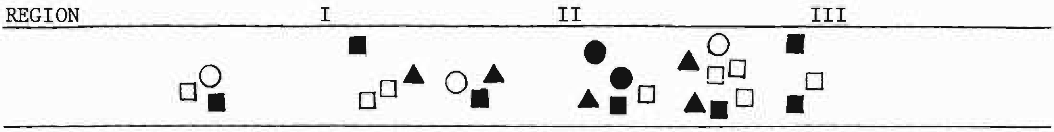

Figure 2. **General distribution of 5 age classes of *E. trivolvis* in the small intestine on day 7 (■), day 14 (□), day 21 (○), day 28 (●), and day 35 (▲) postinfection. Symbol represent the areas in which various age classes of parasites were recovered. Average length of the small intestine was 38 cm.**

Results

Superimposed infections with *E. caproni*

Percent recovery, average number, and total number of worms recovered from superimposed infections with *E. caproni* are summarized in Table 1. Twenty-six of 40 (65%) 7-day-old parasites were present when not challenged by another age class. Clustering, the presence of more than 2 parasites at a site, occurred in *E. caproni* infections. It was possible to establish 5 different age classes in the hamster. Age class distinction was made based on the size and maturation of the worms. Figure 1 depicts the distribution of the 5 age classes within the intestine. Parasites of each age class were found in close association with one another. Site selection occurred based on the preference for the lower two-thirds of the intestine by the parasites. Seven- and 35-day-old *E. caproni* appeared to occupy the same region of the intestine.

No unthriftiness or diarrhea were observed in infected hamsters. At necropsy, ballooning of the small intestine and cecum occurred in hamsters infected with heavy worm burdens. Enlarged lymphatic nodules occurred along the length of the intestine, along with increased vascularization.

Superimposed infections with *E. trivolvis*

Percent recovery, average number, and total number of worms from superimposed infections are summarized in Table 2. The day 21 age class was the dominant age class in the *E. trivolvis* superimposed infections. Day 35 worms occurred in about the same percentage as day 7 worms. It was possible to establish 5 different age classes in the hamster. Figure 2 depicts the distribution of the 5 age classes within the intestine. Parasites of each age class were found in close association with one another. In *E. trivolvis* the 35-day-old age class occupied about half the total intestinal section occupied by the 7-day-old worms.

No unthriftiness or diarrhea were observed in infected hamsters. At necropsy, ballooning of the small intestine and cecum occurred in hamsters infected with heavy worm burdens. Enlarged lymphatic nodules occurred along the length of the intestine, along with increased vascularization.

The effect of a primary *Echinostoma caproni* infection on subsequent challenge with *Echinostoma trivolvis*

No resistance could be demonstrated when a challenge was made 14 days following the primary infection. The primary infection did not appear to be reciprocally influenced by the challenge infection. As illustrated in Figure 3, worms of both species were found closely associated with one another. Table 3 reports the average number of worms recovered and the percent infectivity for this experiment. A difference was noted in the percent infectivity for the 2 species in the concurrent infection. Greater infectivity occurred in the single infection.

The effect of a primary *Echinostoma trivolvis* infection on subsequent challenge with *Echinostoma caproni*

No resistance could be demonstrated when a challenge was made 14 days following the pri-

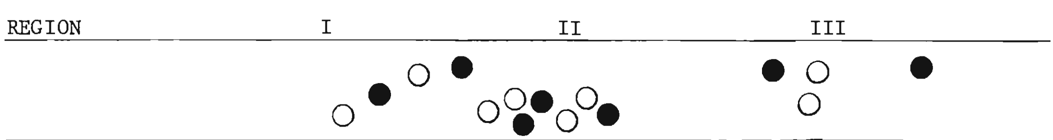

Figure 3. **General distribution of parasites from a primary *E. caproni* infection followed by a challenge with *E. trivolvis*. *E. caproni* day 28 (●), *E. trivolvis* day 14 (○). Symbols represent the areas in which the two age classes of parasites were recovered. Average length of the small intestine was 38 cm.**

Table 3. The average number of worms and infectivity in hamsters infected with *E. caproni* followed by an *E. trivolvis* infection. Results from single infections are also listed.

	E. caproni/E. trivolvis	*E. caproni*	*E. trivolvis*
Mean no. of worms recovered ± SE	5.7 ± 1.7/4.8 ± 1.1	7.6 ± 0.54	6.2 ± 1.1
Percent infectivity	57/48	76	62

mary infection. The primary infection did not appear to be reciprocally influenced by the challenge infection. As illustrated in Figure 4, worms of both species were found in close association with one another. A difference was noted in the percent infectivity for the 2 species in the concurrent infection. The infectivity was similar when the 2 species occurred singly. Table 4 reports the average number of worms recovered and the percent infectivity.

Discussion

The susceptibility to a superimposed *E. caproni* and *E. trivolvis* infection in hamsters in this study differs from the previous findings by Sirag et al. (1980) and Christensen et al. (1986). Their studies involved the resistance of mice to superimposed infections. Christensen et al. (1990) reported on the establishment, survival, and fecundity in *E. caproni* infections in hamsters. They reported that the hamster has a limited capacity to expel primary infections and to mount a regulatory response to superimposed challenge worm establishment. In our study, a cellular immune response occurred. Lymphatic nodules were enlarged and increased vascularization was present in the small intestine. A humoral response may be lacking as evidenced by the survival of the multiple age classes of parasite. This agrees with the study of Mabus et al. (1988) in which no antibody was detected in the serum of golden hamsters infected with *E. trivolvis*.

The results from the present study show a high level of compatibility in the *E. caproni, E. trivolvis*/hamster model. It was possible to establish 5 age classes for both *E. caproni* and *E. trivolvis* in the golden hamsters. The model also is characterized by the ability to superimpose 1 species on the other after establishing 1 infection. In the superimposed infections with *E. caproni,* 26 of 40 (65%) of the 7-day-old worms survived when unchallenged by other age classes, whereas a range of 8–17 (37%) 7-day-old worms survived when other age classes were present. The number of 7-day-old survivors of *E. trivolvis* was the same, 26%, whether challenged or not.

The survival of the 14-day-old worm class also was reduced in the multi-age class infections with *E. caproni,* but unaffected in the study using *E. trivolvis.* Percent recoveries for the 28- and 35-day-old age classes in *E. caproni* were similar. The percent recovery for *E. trivolvis* decreased from a high of 94% for day 21 to 78% for day 28 and 68% for day 35.

A difference between the species occurs as they mature. The 7-and 35-day-old *E. caproni* occupy the same region of the intestine. The young and old worms do not differ in habitat selection. However, in *E. trivolvis* the 35-day-old age class occupies about half the total intestinal section occupied by the 7-day-old worms. Thus, this species may become more specific in habitat selection as it matures.

The results seem to indicate that intraspecific competition occurred in *E. caproni* with the 7- and 14-day-old age classes and in *E. trivolvis* with the 21-, 28-, and 35-day-old age classes. According to Kisielowska (1970), parasites within the same host organism may interact negatively resulting in a decrease in the number of surviving parasites. Indirect effects of the immunologically mediated inflammatory reaction or worm crowding may be possible mechanisms for the results obtained.

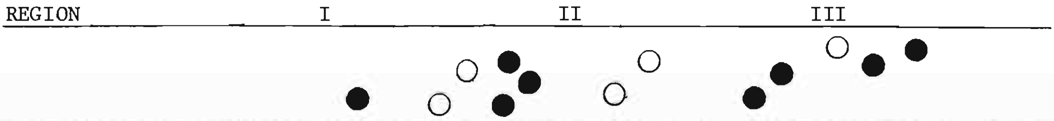

Figure 4. General distribution of parasites from a primary *E. trivolvis* infection followed by a challenge with *E. caproni. E. trivolvis* day 28 (○), *E. caproni* day 14 (●). Symbols represent the areas in which the two age classes of parasites were recovered. Average length of the small intestine was 38 cm.

Table 4. The average number of worms and percent infectivity in hamsters infected with *E. trivolvis* followed by an *E. caproni* infection. Results from single infections with each parasite are also listed.

	E. trivolvis/E. caproni	*E. caproni*	*E. trivolvis*
Mean no. of worms recovered ± SE	4.6 ± 1.1/6.7 ± 0.94	6.1 ± 1.3	4.8 ± 0.89
Percent infectivity	46/67	61	48

Huffman et al. (1988) infected golden hamsters simultaneously with *E. caproni* and *E. trivolvis.* In that study, concurrent infections with *E. trivolvis* differed from single ones in that there was a tendency for the parasites to move from the ileum to the jejunum. The number of *E. caproni* recovered in concurrent infections was markedly reduced. Fried and Gainsburg (1980) reported a reduced recovery of *Notocotylus* sp. in the presence of *Zygocotyle lunata.* Holmes (1961) found that worm distribution in the host gut was affected by concurrent infections with *Moniliformis dubius* and *Hymenolepis diminuta.*

In the present concurrent study the parasites were not administered simultaneously but at an interval of 2 weeks between primary and challenge infections. This study is in agreement with Huffman et al. (1988) in that worms of both species were found in clusters or in close association with each other. The general findings from the present study indicate that this model is useful for elucidating aspects of intra- and interspecific interactions.

Acknowledgments

This work was supported in part by a grant from the East Stroudsburg University Faculty Development and Research Committee. We gratefully thank C. Dean Miller for typing the manuscript.

Literature Cited

Behnke, J. M. 1987. Evasion of immunity by nematode parasites causing chronic infections. Parasitology 26:2–71.

Christensen, N.Ø., J. Knudsen, and J. Andreassen. 1986. *Echinostoma revolutum*: resistance to secondary and superimposed infection in mice. Experimental Parasitology 61:311–318.

———, P. Nansen, B. Ø. Fagbemi, and J. Monrad. 1987. Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infections of mammalian hosts. Parasitology Research 73:387–410.

———, P. E. Simonsen, A. B. Odaibo, and H. Mahler. 1990. Establishment, survival, and fecundity in *Echinostoma caproni* (Trematoda) infections in hamsters and jirds. Journal of the Helminthological Society of Washington 57:104–107.

Dobson, A. P. 1985. The population dynamics of competition between parasites. Parasitology 91: 317–347.

Franco, J., J. E. Huffman, and B. Fried. 1986. Infectivity, growth and development of *Echinostoma revolutum* (Digenea: Echinostomatidae) in the golden hamster, *Mesocricetus auratus.* Journal of Parasitology 72:142–147.

Fried, B., and D. M. Gainsburg. 1980. Concurrent infections of cecal trematodes *Zygocotyle lunata* and *Notocotylus* sp. in the domestic chick and observations on host-parasite relationships of *Notocotylus* sp. Journal of Parasitology 66:502–505.

Holmes, J. C. 1961. Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala): general effects and comparison with crowding. Journal of Parasitology 47:209–216.

———. 1990. Competition, contacts, and other factors restricting niches of parasitic helminths. Annales de Parasitologie Humaine et Compareé 65: 69–72.

Huffman, J. E., C. Michos, and B. Fried. 1986. Clinical and pathological effects of *Echinostoma revolutum* (Digenea: Echinostomatidae) in the golden hamster, *Mesocricetus auratus.* Parasitology 93: 505–515.

———, A. Alcaide, and B. Fried. 1988. Single and concurrent infection of the golden hamster, *Mesocricetus auratus,* with *Echinostoma revolutum* and *E. liei* (Trematoda: Digenea). Journal of Parasitology 74:604–608.

Isaacson, A. C., J. E. Huffman, and B. Fried. 1989. Infectivity, growth, development and pathology of *Echinostoma caproni* (Trematoda) in the golden hamster. International Journal for Parasitology 19: 943–944.

Kanev, I. 1985. On the morphology, biology, ecology and taxonomy of *E. revolutum* group (Trematoda: Echinostomatidae: *Echinostoma*). Doctoral Dissertation, University of Sofia, Bulgaria.

Kisielowska, K. 1970. On the theoretical foundations of parasitosynecology. Bulletin of The Polish Academy of Sciences. Biological Science Series 18: 103–106.

Mabus, J., J. E. Huffman, and B. Fried. 1988. Humoral and cellular response to infection with *Echinostoma revolutum* in the golden hamster, *Mesocricetus auratus.* Journal of Helminthology 62: 127–137.

Odaibo, A. B., N. Ø. Christensen, and F. M. A. Ukoli.

1988. Establishment, survival and fecundity in *Echinostoma caproni* infections in NMRI mice. Proceedings of the Helminthological Society of Washington 55:265–269.

Sirag, S. B., N. Ø. Christensen, F. Frandsen, J. Monrad, and P. Nansen. 1980. Homologous and heterologous resistance in *Echinostoma* infections in mice. Parasitology 80:479–486.

MEETING NOTICES

The Second International Symposium on Monogenea will be held in Montpellier, France, 5–8 July 1993. For information contact:

Dr. Alain Lambert
Laboratoire de Parasitologie Comparée C. C. 105
Université des Sciences et Techniques de Languedoc
Place E. Bataillon–34095
Montpellier Cédex 05, FRANCE

The Twenty-first International Nematology Symposium will be held in Albufeira, Portugal, 12–17 April 1992. For information contact:

Secretary 21st International Nematology Symposium
% Departmento de Zoologia
Universidade de Coimbra
3049 Coimbra Codex, PORTUGAL

The Eighth International Conference on Trichinellosis will be held in Herceg Novi, Montenegro, Yugoslavia, 22–26 September 1992. For information contact:

Dr. Kosta Cuperlovic
INEP
Banatska 31B
11080 Zemun, YUGOSLAVIA